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THE DETECTION OF A NEW SPECIFIC ANTIGEN IN CELLS INFECTED WITH MYXOVIRUSES

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We previously reported that immune rabbit sera displayed high virus inhibiting activity with respect to the chorioallantoic membrane of a chick embryo infected with Newcastle disease virus and smallpox vaccines in prevention and treatment experiments on chick embryos. It was impossible to explain the sera's high effectiveness against infected tissues by simple summation of the effect of antiviral and antitissue antibodies since a mixture of antiviral and antitissue sera did not produce a similar therapeutic effect [4].

It could be assumed that antibodies against new antigenic components, products of the virus' interaction with sensitive cells of the membrane, appear in the sera against the infected tissues.

There are reports about the development of a new antigen in tumor cells of animal and human origin transformed by SV40 viruses [9, 12, 17, 19], by polyoma virus [11, 21], by types 7, 12, 18 [14, 15], as well as by Rous sarcoma virus [13].

There is information concerning the appearance of a new cellular antigen in tissues infected with other animal viruses: rabies and poliomyelitis [1, 2], herpes [18], type 5 adenovirus [10], as well as bacterial viruses [20].

We studied the possibility of the development of new antigenic components in tissues infected with myxoviruses.

MATERIAL AND METHODS

Allantoic cultures of Newcastle disease virus (Strain EPM-1) and influenza Type A virus (Strain PR8) which were passed many times through chick embryos were used. The hemagglutinating titers of the viruses were 1:640 and 1:1280. The infectious titers were

10^8 - 10^9 EID₅₀ for the MDV (NDV) virus and 10^6 - 10^7 EID₅₀ for PR8. The infectious titer of the PR8 virus (allantoic strain) on mice was $10^{3.5}$.

Immune rabbit sera against the chorioallantoic membrane of a chick embryo infected with Newcastle disease virus (KHAOZ No. NDV) was obtained according to a system described previously [36]. Mouse sera against lungs infected with PR8 virus were obtained by bleeding the animals on the 14th-16th day after their infection with sublethal doses equal to 10 - 100 LD₅₀.

The immune rabbit sera was adsorbed with formalinized chorioallantoic membrane tissue (KHAON) and with NDV virus adsorbed on formalinized chick erythrocytes [6, 7].

The sera of mice which had undergone an influenza infection were freed from virus antibodies by adsorption both with a native eluate of PR8 virus and with eluate disintegrated with ether. Formalinized human erythrocytes were charged with V and S antigens. The treatment of the virus eluate with ether was carried out by Leif and Henle's method [16].

In order to eliminate the anticomplementary nature of the adsorbed sera which develops in connection with the transformation of the antibody-virus complexes in them, the sera were centrifuged at 35,000 rpm for 1 hour at $2-4^\circ$. Before setting up the complement fixation test the sera were heated at 56° for 30 min.

In order to detect antigenic changes in the sensitive cells from their interaction with myxogroup viruses, 20% water-salt extracts of normal and infected (taken 24 hours from the moment of infection with the viruses) chick embryo membranes were studied in a complement fixation test. In addition, extracts of normal tissues and the lungs of mice killed on the 6th-8th day after infection with PR8 virus, as well as virus eluates [5] and V and S antigens [5, 16] were used in the reaction.

The titers of viruses in the extracts of the infected membranes in the hemagglutination reaction were 1:320-1:1280. Hemagglutinins were not found in the infected lungs (6th-7th-8th day) evidently as a result of their suppression by tissue inhibitors and antibodies.

The amount of protein in the tissue extracts was determined by Lauri's method and varied from 2.708 to 3.916 mg/ml.

The serological reactions (hemagglutination, antihemagglutination and complement fixation reaction) were set up according to methods described earlier [8].

RESULTS

The results of a serological study of sera of rabbits immunized against the chorioallantoic membrane of a chick embryo infected with NDV virus are presented in Table 1.

TABLE 1

Detection of New Complement Fixing Antigen in Chorioallantoic Membrane Tissue Infected with Newcastle Disease Virus

1 Исходная сыворотка пробы	2 Титр антител (1:1000)	3 Абсорбент сыворотки	4 Разведение сыворотки	5 РСК с антигенами				11 Контроль гемолитика
				6 из вируса NDV	7 из тканей	9 XAOH	10 XAOZ	
				инфицирован- ная алланто- ическая жидкость	у.	с.		
1 2 К оболочке, инфици- рованной вирусом	1:10	1 4 До адсорбции	1:40 1:80 1:160 1:320 1:640 1:80 1:160 1:320 1:40 1:80 1:160 1:40 1:80 1:160	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	— — — — — — — — — — — — — — —
3 3 Те же	—	1 5 Ткань XAOH	1:40 1:80 1:160 1:320 1:40 1:80 1:160 1:320 1:40 1:80 1:160 1:40 1:80 1:160	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	— — — — — — — — — — — — — — —
» »	0	1 6 Вирус	1:40 1:80 1:160 1:320 1:40 1:80 1:160 1:320 1:40 1:80 1:160 1:40 1:80 1:160	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	— — — — — — — — — — — — — — —
» »	0	1 7 Ткань XAOH и вирус	1:40 1:80 1:160 1:320 1:40 1:80 1:160 1:320 1:40 1:80 1:160 1:40 1:80 1:160	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	— — — — — — — — — — — — — — —

Note: Notation here and in Tables 2 and 3: +++, ++, + (+) degree of inhibition of erythrocyte hemolysis; — complete hemolysis of erythrocytes; . reaction not set up.

1) Immune rabbit serum; 2) titer of antihemagglutinins; 3) serum adsorbent; 4) serum dilution; 5) complement fixation reaction with antigens; 6) from NDV virus; 7) from tissues; 8) infected allantoic fluid; 9) XAOH; 10) XAOZ; 11) control serum; 12) against membrane infected with virus; 13) the same; 14) before adsorption; 15) XAOH tissue; 16) virus; 17) XAOH tissue and virus.

TABLE 2

Complement Fixing Antibodies Against New Antigen Developing in Mouse Lungs During Experimental Influenza (PR8)

1 Исходные титры	2 Титр антиген- ной сыворотки мыш (P10A)	3 Адсорбент	4 Разведение сыворотки	5 РСК с антигенами					11 Контроль сыворотки
				6 вируса гриппа PR 8		7 эритроциты мышь	8 нормальная мышь	9 инфицированная мышь	
				8 элюат	9 V.				
2	1:320	1 2 До адсорбции	1:20	+++	+++	+++	+++	+	+
			1:40	+++	+++	+++	+++	+	+
			1:80	+++	+++	+++	+++	+	+
			1:160	++(+)	+++	+++	+++	+	+
2	0	1 3 Элюат PR8	1:40	---	---	---	---	---	---
			1:80	---	---	---	---	---	---
			1:160	---	---	---	---	---	---
			1:40	---	---	---	---	---	---
6	1:320	1 2 До адсорбции	1:20	+++	+++	+++	+++	+	+
			1:40	+++	+++	+++	+++	+	+
			1:80	+++	+++	+++	+++	+	+
			1:160	+++	+++	+++	+++	+	+
6	0	1 4 Элюат PR8, дезинтегриро- ванный эфиром	1:20	---	---	---	---	---	---
			1:40	---	---	---	---	---	---
			1:80	---	---	---	---	---	---
			1:160	---	---	---	---	---	---

Note: The sera were taken on the 14th day after infection.

1) Number of serum; 2) antihemagglutinin titer; 3) adsorbent; 4) serum dilution; 5) complement fixation reaction with antigens; 6) of PR8 influenza virus; 7) lung extract; 8) eluate; 9) normal mouse; 10) infected mouse; 11) control serum; 12) before adsorption; 13) PR8 eluate; 14) PR8 eluate disintegrated with ether.

TABLE 3

Specificity of New Complement Fixing Antigen Developing in Chorioallantoic Membrane Tissue of Chick Embryo from the Effect of Myxoviruses

1 Исходная проба	2 Абсорбент сыворотки	3 Разведение сыворотки	4 РСК с антигенами							Контроль сыворотки																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
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12 к сыв. проб. сыв.	14 До абсо- рбции	1:40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

1) Immune rabbit serum; 2) serum adsorbent; 3) serum dilution; 4) complement fixation reaction with antigens; 5) from viruses; 6) from tissues; 7) infected allantoic fluid; 8) Sendai; 9) KHAON; 10) KHAOZ; 11) control serum; 12) No. 51 against membrane infected with virus; 13) the same; 14) before adsorption; 15) KHAON and virus.

It is seen from Table 1 that the sera of rabbits immunized against membrane infected with NDV virus produced a positive reaction both with antigens from normal and infected membranes and with virus antigens (allantoic fluid and V and S antigens). The intensity of complement fixation with KhaON and KhAOZ antigens was approximately the same.

The experiments on separate specific adsorption showed that normal membrane tissue completely eliminated from the serum antibodies against normal membrane antigens. Adsorption of the serum with virus led to the elimination of antiviral antibodies and partially of antitissue antibodies evidently because of the common features of the virus antigens and the host's cells [5,8].

As a result of successive multiple adsorption first with KhaON tissue and then with virus preparation the serum did not react either with infected allantoic fluid or with KhaON extract, as well as with components of the virus particle - V- and S-antigens. However, sera devoid of antiviral antibodies and antibodies against antigens of normal chorioallantoic membrane continued to fix complement in the presence of an extract of infected membrane.

On the basis of these experiments we came to a conclusion concerning the development in chorioallantoic membrane infected with virus of a new specific antigen which differs both from virus antigens and from normal membrane antigens.

It also seemed of interest to determine whether new antigenic components develop in mouse lungs from an experimental influenza infection, that is, whether it is possible to detect in mice which underwent an influenza infection antibodies against products of the interaction of virus and cells which arose in the course of the infection.

For this purpose, from mice infected with sublethal doses of PR8 virus, at the height of the disease (6th, 7th and 8th days after infection) the infected lungs were taken, from which antigens were later prepared for the complement fixation reaction.

The mice which survived were bled on the 14th-16th day after infection and their sera tested in the complement fixation reaction and the antihemagglutination reaction.

As seen from the results of the investigations presented in Table 2, the sera of variolated mice reacted rather intensively both with viral antigens (eluate of PR8 virus, V- and S- viral antigens) and with infected lung tissue but did not fix complement with an extract of normal lung tissue.

Adsorption of the mouse sera with an eluate of PR8 virus led to the removal of antibodies against viral eluate and V-antigen, but the reaction with S-antigen and an extract of infected lung continued. It was only possible to completely remove antiviral antigens (anti-V and anti-S) by adsorbing the sera with an eluate of PR8 virus disintegrated with ether and adsorbed on formalinized chick erythrocytes. The adsorbed sera in the complete absence of a reaction with viral antigens specifically fixed complement only in the presence of antigen from an infected lung.

Of the ten samples of sera (each of which was a mixture of blood from 3-4 mice) only 3 after adsorption reacted with antigen from infected lungs.

Thus, these experiments showed that antibodies against the antigen of lung tissue infected with virus appear in mice infected with influenza virus (PR8). This antigen differs in specific properties both from viral antigens (viral eluate, V- and S- antigens) and from antigens of normal lung tissue.

Our experiments showed that the specific antigen arises in infected cells not only under the influence of influenza virus (PR8) but also as a result of infecting a chick embryo membrane with other myxogroup viruses. The results of the experiments are presented in Table 3. The positive reaction of sera against infected membrane with antigens from membranes infected with various representatives of the myxoviruses, in the complete absence of a reaction with viral preparations and an extract of an uninfected membrane, indicates the similarity of the antigenic changes arising in the chick embryo membrane under the influences of the viruses which we investigated.

Our data differ somewhat from the results obtained by investigators with oncogenic viruses in which the appearance of a new antigen is determined by the species affiliation of the virus. It has not been excluded that the viruses of the myxogroup studied by us which are similar in certain properties also caused the appearance of similar specific antigens in the infected tissues of the chick embryo. This question needs further investigation.

The experiments on the chemical nature of the specific antigen of the infected membrane showed that the antigen completely loses serological specificity under the influence of trypsin and boiling. Heating at 56° for 30 min and potassium periodate decreased the new antigen's activity only to a small degree. It is clear that the specificity of the new cellular antigen is connected with the protein.

CONCLUSIONS

1. New antigens develop in chorioallantoic membrane tissue infected with Newcastle disease virus which are evidently of a protein nature and differ serologically both from normal host cells and from viral antigens.
2. During an influenza infection antibodies appear in mice which react specifically only with antigen from an infected lung, which indicates the appearance in infected lung cells of an antigenic component heterologous for the animal organism.
3. The new antigens developing in infected cells under the influence of viruses of the myxogroup (NDV, PR8 and Sendai) did not differ in specific serological properties.

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DETECTION OF A NEW SPECIFIC ANTIGEN IN CELLS INFECTED WITH MYXOVIRUSES

P. N. Kosyakov, T. A. Posevaya, Z. I. Rovnova

Summary

Serological investigations aimed at detection of new antigenic components in tissues affected by myxoviruses were carried out. It was shown that in the tissue of the chorio-allantoic membrane of chick embryos infected with Newcastle disease virus and in the lungs of mice infected with influenza A PR8 virus new specific antigens appear differing both from the viral antigens and from antigens of normal cells. New antigens induced by Newcastle disease virus, influenza A-PR8 and parainfluenza Sendai viruses did not differ serologically.